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(71) Applicant: NOVO NORDIK A/S [DK/DK]; Novo Allé, DK-2880 Bagsværd (DK).

(72) Inventor: CHRISTENSEN, Thorkild; 55 Bellisvej, DK-3450 Alleroed (DK).

(74) Agent: NOVO NORDISK A/S; Corporate Patents, Nilausen, Kim, Novo Allé, DK-2880 Bagsværd (DK).

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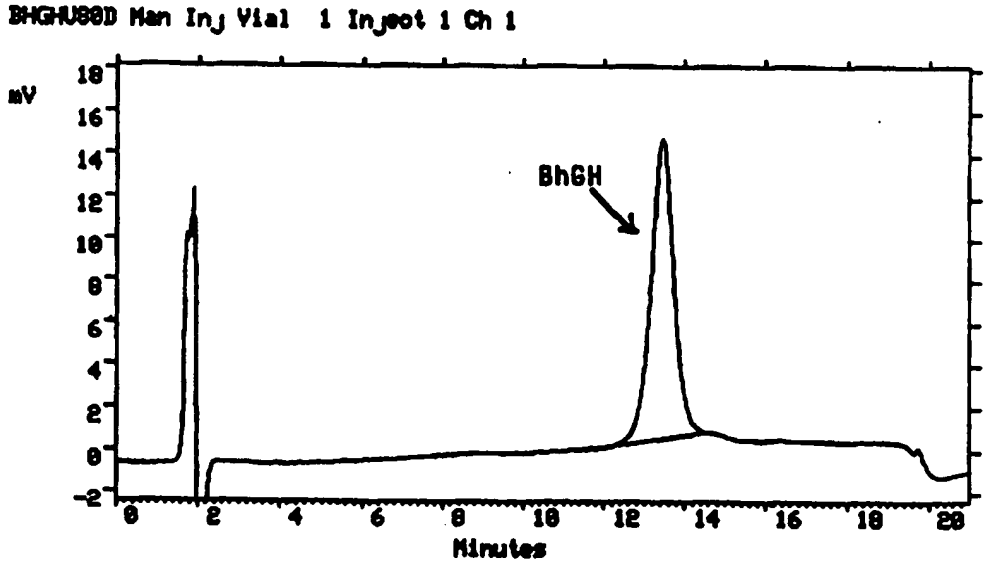
(54) Title: A METHOD OF CONVERTING A HYDROPHOBIC DERIVATIVE OF A POLYPEPTIDE INTO THE NATIVE FORM



(57) Abstract

A method of treating a polypeptide. A hydrophobic derivative of a growth hormone may be treated with a sulfite compound for converting the derivative into the native form of the growth hormone. Preferably, the growth hormone is human growth hormone, and the sulfite is sodium sulphite in a concentration of 20 mM.

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TITLE

A method of converting a hydrophobic derivative of a polypeptide into the native form.

FIELD OF THE INVENTION

The present invention relates to a method of converting a hydrophobic derivative of a polypeptide into the native form especially a method of converting a hydrophobic derivative of a growth hormone into the native form of the growth hormone.

BACKGROUND OF THE INVENTION

The growth hormones from man and from the common domestic animals are proteins of approximately 191 amino acids, synthesized and secreted from the anterior lope of the pituitary gland. Human growth hormone consists of 191 amino acids having a molecular weight of 22125 D. Four cystein residues are present giving rise to two disulfide bridges. The disulfide bridge formed between Cys(53) and Cys(165) results in a major loop, and the disulfide bridge between Cys(182) and Cys(189) results in a minor loop.

Growth hormone is a key hormone involved in the regulation of not only somatic growth, but also in the regulation of metabolism of proteins, carbohydrates and lipids.

The organ systems affected by growth hormone include the skeleton, connective tissue, muscles, and viscera such as liver, intestine, and kidneys.

Until the development of the recombinant technology and the cloning of the growth hormone gene now giving rise to production of e.g. human growth hormone (hGH) and Met-hGH in industrial scale, human growth hormone could only be obtained by extraction from the pituitary glands of human cadavers.

The very limited supplies of growth hormone restricted the use thereof to longitudinal growth promotion in childhood and puberty for treatment of dwarfism, even though it has been proposed for inter alia treatment of short stature (due to growth hormone deficiency, normal short stature and Turner syndrom), growth hormone deficiency in adults, infertility, treatment of burns, wound healing, dystrophy, bone knitting, osteoporosis, diffuse gastric bleeding, and pseudoarthrosis.

Furthermore, growth hormone has been proposed for increasing the rate of growth of domestic animals, for decreasing the proportion of fat in animals to be slaughtered for human consumption, and for increasing the production of milk in lactating animals.

In recombinant techniques human growth hormone is normally produced by expressing a gene coding for human growth hormon, said gene being inserted into a microorganism. The growth hormone is then isolated from the broth, optionally after lysing the microorganisms. The host most commonly used for expressing hGH is E. coli.

hGH extracted from pituitaries have been investigated in order to detect aberrant forms and determine their specific activities. Besides the growth hormone with a molecular weight as mentioned above a variant single chain form is also produced, wherein the amino acid residues 32-46 are omitted resulting in the socalled 20k form of hGH. This variant is the result of alternate splicing at the m-RNA level. Also variants related to mass, charge, rearrangements, oxidized forms, and split forms are described to be present in hGH-preparations isolated from pituitary glands.

The development of new assays has enabled detection of derivatives of growth hormone present in very small amounts in preparations and standards.

In Eur. J. Biochem.(1994), 219, 365-373 is disclosed a trisulphide derivative of biosynthetic human growth hormone produced in Escherichia coli as well as a method of transformation of this derivative into native biosynthetic human growth hormone.

The hydrophobic impurity was detected in connection with the purification of human growth hormone formulations using Hydrophobic Interaction Chromatography (HIC) under special conditions. This derivative is normally not detected by any of the other methods employed for testing a sample of human growth hormone including SDS-PAGE, RP-HPLC, IE-HPLC and GPC or by the HIC method run under other conditions.

For preparing pharmaceutical formulations it is generally preferred to employ active ingredients in a form as pure as possible and, if possible, it is preferred to employ active ingredients being monocomponent compounds.

Eur. J. Biochem.(1994), 219, 365-373 discloses a method of transformation of the hydrohpobic derivative into native biosynthetic human growth hormone by treatment with cysteine.

It is desirable to have the option to use various methods for removing the derivatives from a batch of growth hormone and especially methods wherein is used reagents being readily obtainable and being as safe as possible to handle.

It is possible to remove the hydrophobic derivative by physical separation techniques. However, such a procedure alone is less desirable due to loss of active ingredient.

BRIEF DESCRIPTION OF THE INVENTION

It has surprisingly been found that the hydrophobic derivative of human growth hormone may easily be converted

into the native form of human growth hormone by treating the derivative with a sulphite compound.

Accordingly, the present invention relates to a method for converting the hydrophobic derivative of a growth hormone into the native form of the growth hormone by treating the derivative with a sulphite compound. The treatment is conveniently carried out in a solution comprising the growth hormone in a solvent.

It has been found that the hydrophobic derivatives may be converted directly into the native form by a gentle treatment using a sulphite compound. Thus, the conversion or "refolding" may according to the invention be carried out using a conventional buffer for refolding of proteins, but without the preceding reduction or denaturation to break the disulfide bridges normally relied upon when refolding proteins.

The sulphite compound to be used in accordance with the present invention mey be an alkali metal sulphite such as sodium sulphite or potassium sulphite, ammonium sulphite, or an alkaline-earth metal sulphite such as magnesium sulphite or calcium sulphite.

In accordance with another aspect of the invention, the sulphite compound is generated in situ in a solution for the treatment of the hydrophobic derivative of growth hormone by adding sulphur dioxide to the solution, preferably by bubbling sulphur dioxide through the solution.

The concentration of the sulphite compound in the reaction may according to the invention be up to a saturated solution, preferably from 1 to 30 mM, and most preferred about 20 mM.

In a preferred embodiment of the invention, a hydrophobic derivative of hGH is treated with sodium sulphite in a concentration of about 20 mM at pH 7 and at room tempreature.

The hydrophobic derivative of growth hormone may optionally be isolated before carrying out the conversion thereof into the corresponding native growth hormone.

It is preferred to treat the whole batch of growth hormone found to comprise the hydrophobic derivative of GH directly without isolating the growth hormone derivative.

The solvents used to prepare the solution of derivative of the growth hormone to be treated may e.g. be an aquous buffer buffered at a pH from 3 to 11. Solutions being buffered to a pH > 6 are preferred, and more preferred are solutions buffered to about pH 7. The solvent is preferably selected from the group consisting of Tris, triethylamine, citric acid, phosphate buffer, and histidine.

A preferred solution is has pH 7.0.

The method of the invention is carried out at temperatures ensuring a suitable rate of conversion of the hydrophobic derivative and a minimum formation of unwanted side products from degradation of the growth hormone. Normally the reaction is carried out at temperatures from the freezing point to about 50°C, preferably at a temperature from about 5°C to about 30°C, more preferred form about 15°C to about 25°C, and most preferred at room temperature.

Presence of the hydrophobic derivative of a growth hormone comprising an extra sulphur atom as compared to the native growth hormone wherein the growth hormone may be detected by a hydrophobic interaction chromatography wherein the column is eluted using a gradient of ammonium sulphate and detecting the presence of the hydrophobic derivative.

Hydrophobic interaction chromatography is inter alia described in LC&GC.INTL Vol. 5, No. 11 (1992) 24-29.

The HIC may be carried out using a column of phenyl superose in a FPLC apparatus. A convenient apparatus is the FPLC apparatus Phenyl Superose HR 5/5 offered by Pharmacia.

The elution may be carried out using suitable salts such as ammonium sulphates and/or ammonium acetate.

The fractions of the eluate from the HIC comprising the hydrophobic derivative of growth hormone may then be subjected to peptide mapping as disclosed in Chapter 9 in High Performance Liquid Chromatography in Biotechnology, Edited by William S. Hancock, Published by John Wiley & Sons, Inc, 1990.

The hydrophobic derivative of growth hormone may be detected by comparing retention times as the fragment comprising a trisulphide bridge has a longer retention time as compared to the corresponding fragment comprising disulphide bridge.

The native growth hormone prepared in accordance with the present invention may be used for preparing pharmaceutical formulations in the form of e.g. a solution or a lyophilized powder in any method known per se, e.g. as disclosed in International Patent Publications Nos. WO 89/09614, WO 93/12811, WO 93/12812, WO 93/19776, or WO 94/03198 or in published European patent applications Nos. EP 303746 or EP 374120.

The growth hormone prepared in accordance with the present invention may be used for preparing pharmaceutical formulations formulated for administration in any suitable way, e.g. by parenteral or oral administration or administration to a mucosal membrane, e.g. nasal, buccal, sublingual, or vaginal administration. The pharmaceutical

formulation may be presented in the form of a dose comprised in a vial or cartridge or any other suitable container such as a prefilled syringe or a pen device.

Thus, the growth hormone may be formulated in the form of a lyophilized powder to be reconstituted later using conventional vehicles such as distilled water or water for injection or in the form of a solution comprising growth hormone and a solvent.

The solvent used may be water, alcohols such as ethyl, n-propyl or isopropyl, butyl alcohol or mixtures thereof.

Such pharmaceutical formulations may furthermore comprise salts for adjusting the tonicity, buffers for adjusting the pH and optionally an excipient in order to facilitate the processing thereof, e.g. lyophilization and the the rapid and complete dissolution of a lyophilized formulation when reconstituting the formulation before use. The formulations may comprise conventional preservatives such as phenol, benzyl alcohol, m-cresol, methyl paraben, propylparaben, benzalconium chloride, and benzethonium chloride.

A buffer may e.g. include phosphate, tris, citrate, succinate, acetate, histidine or asparagine buffers.

Suitable pH ranges, adjusted with buffer, for hGH formulations are from about 4 to about 8, more preferably from about 5.5 to about 7, most advantageously about 6.0.

An excipient may be selected saccharids such as lactose, trehalose, and sucrose, sugar alcohols such as sorbitol, xylitol, ribitol, myoinisitol, galactitol, mannitol or the like , polysaccharides such as the polymers commercialized as Dextran® products such as Dextran® 40, Dextran® 70 or Dextran® 75, and Ficoll® and polyvalent alcohols such as

polyethylene glycol or polyvinyl alcohol or a combination of two or more of these.

Neutral salts such as sodium chloride or potassium chloride are optionally used in place of sugars or sugar alcohols.

In the present context "growth hormone" may be growth hormone from any origin such as avian, bovine, equine, human, ovine, porcine, salmon, trout or tuna growth hormone, preferably bovine, human or porcine growth hormone, human growth hormone being most preferred. The growth hormone to be treated in accordance with the present invention may be growth hormone isolated from a natural source, e.g. by extracting pituitary glands in a conventional manner, or a growth hormone produced by recombinant techniques, e.g as described in E.B. Jensen and S. Carlsen in Biotech and Bioeng. 36, 1-11 (1990). The preferred growth hormone is hGH.

The "growth hormone" may also be a truncated form of growth hormone wherein one or more amino acid residues has (have) been deleted; an analogue thereof wherein one or more amino acid residues in the native molecule has (have) been substituted by another amino acid residue, preferably a natural amino acid residue, as long as the substitution does not have any adverse effect such as antigenicity or reduced action; or a derivative thereof, e.g having an N- or C-terminal extension such as Met-hGH, Met-Lys-hGH, Ala-Glu-hGH, Thr-Glu-Ala-Glu-hGH, Ala-Glu-Ala-Glu-hGH, Met-Glu-Ala-Glu-hGH, Met-Phe-Glu-Glu-hGH, Met-Asp-Ala-Asp-hGH, or Met-Glu-Ala-Asp-hGH.

The term "dose" of growth hormone refers to that amount that provides therapeutic effect in an administration regimen. The formulations hereof are prepared containing amounts of hGH at least about 0.1 mg/ml, preferably upwards of about 10 mg/ml, preferably from about 1 mg/ml to about 40 mg/ml, more preferably from about 1 mg/ml to about 25 mg/ml, e.g. from 1 mg/ml to about 5 mg/ml, calculated on the ready-to-use formulation.

For use of these compositions in administration to human beings suffering from hypopituitary dwarfism, for example, these formulations contain from about 0.1 mg/mg to about 10 mg/ml, corresponding to the currently contemplated dosage regimen for the intended treatment. The concentration range is not critical to the invention and may be varied by the physician supervising the administration.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is described more in detail with reference to the drawings in which

Fig.1 shows a HIC chromatogram for determination of BhGH' in a sample treated with a 1mM solution of sodium sulphite, and

Fig.2 shows a HIC chromatogram for determination of BhGH' in a sample treated with a 20mM solution of sodium sulphite.

DETAILED DESCRIPTION OF THE INVENTION

The identity of amino acid sequence of the hydrophobic variant of human growth hormone with that of human growth hormone has been determined by tryptic peptide mapping, amino acid sequence analysis of isolated peptide fragments. Furthermore, mass spectrometry has been carried out.

The mass spectrometry showed an increase of mass of 32 daltons of the hydrophobic derivative of hGH as compared to native hGH. This can be assigned to the presence of an extra sulphur atom.

From the results of the characterization of the hydrophobic growth hormone derivative it was concluded that the derivative is a human growth hormone having one disulphide bridge (Cys 53-Cys 165) and one trisulphide bridge (Cys 182 - S - Cys 189) and having an amino acid sequence identical to that of native hGH.

EXPERIMENTAL PART

Example A

Detection of Hydrophobic Derivative of Human Growth Hormone.

The presence of a hydrophobic derivative of recombinant human growth hormone comprising an extra sulphur atom as compared to the native form thereof was detected in accordance with the invention by subjecting the growth hormone to HIC using a FPLC apparatus (Pharmacia) and column of Phenyl Superose HR 5/5 from Pharmacia.

For elution a gradient of ammonium sulphate is used.

The buffer system was:

Buffer A: 1.2M ammonium sulphate, 20mM Tris pH 7.4
Buffer B: 20mM Tris pH 7.4

The chemicals used were all Merck p.a.

The elution was carried out using the following gradient:

Time (min.)	Buffer
0.0	Conc %B 0.0
1.0	Conc %B 0.0
10.0	Conc %B 100
16.0	Conc %B 100
17.0	Conc %B 0
22.0	End

The buffer was added at a rate of 0.50 ml/min.

The fractions of the eluate comprising the hydrophobic derivative were subjected to peptide mapping as mentioned above.

Alternative Mehod of Detection of Hydrophobic Derivative of Human Growth Hormone

hGH samples were analyzed on a TSK Ether 5PW (75 x 4.6 mm ID) column at ambient temperature using eluent C and D and a gradient from 40 to 50% eluent D during 30 minutes. Eluent C: 2 M $(NH_4)_2SO_4$, 20 mM Na_2HPO_4 x $2H_2O$, pH 6.0. Eluent D: 20 mM Na_2HPO_4 x $2H_2O$, 0.1% PEG, pH 6.0. Detection was performed at 280 nm. Flow: 0.5 ml/min. HPLC equipment: Data handling and control: Waters 860 Networking computer system, Pumps: Waters pumps model 510, Sample injectors: Waters Wisp 712, Detector: Waters M481 spectrophotometer.

The hydrophobic derivative of recombinant human growth hormone (rhGH') was identified by the appearance of an new peak between peak 8 and peak 9 coupled with the disappearance of peak 7 (the 7 peptide) corresponding to amino acid residues 179-191 in a peptide mapping of recombinant human growth hormone (rhGH). The numbering of the peaks are as disclosed in Chapter 9 in High Performence Liquid Chromatographi (Supra).

Isolation of hydrophobic Derivative of Human Growth Hormone

If it is desired to isolate the hydrophobic derivative from a sample of hGH, such isolation may be carried out by scaling up the procedure described above, or such isolation may e.g. be carried out using the method as described in Bio/Technology 5 (1987) 161-164.

Characterization of Hydrophobic Derivative of Human Growth Hormone by Mass Spectroscopy

Recombinant human growth hormone was analyzed by Plasma Desorption Mass Spectroscopy (PDMS) and Electro-Spray Mass Spectroscopy (ESMS), respectively.

The analysis focused on the detecting the difference between the intact rhGH and rhGH' and the corresponding 7 and 7' tryptic peptides, respectively.

Determination of Mass of Intact rhGH and rhGH'

The mass of intact rhGH and rhGH' was analyzed by ESMS performed using a API III LC/MS/MS system (Sciex. Thornhill, Ontario, Canada). The triple quadropople instrument had a mass-to-charge (m/Z) range of 2400 and was fitted with a pneumatically assisted electrospray (also referred to as ion-spray) interface (P1, P1). Sample introduction was done by a syringe infusion pump (Sage Instruments, Cambridge, MA) thorugh a fused capillary (75 µm i.d.) with a liquid flow rate set at 0.5-1 µl/min. The instrument m/z scale was calibrated with single charged ammonium adduct ions of poly(propylene glycols) (PPG's) under unit resolution. The accuracy of mass determination was in generally better than 0.02%, but low intensity spectra may result in less precise mass determination.

Plasma Desorption Mass Spectometry (PDMS) analysis was performed using a BIO-ION 20K 252-Californian time-of-flight instrument (ABI Nordic A/S, Uppsala, Sweden). Standard procedures for sample application (including in situ reduction using DTT) and analysis were followed (P3,P4). The accuracy of Mass determination was about 0.1%.

Before the analysis, both rhGH and rhGH' were desalted. The rhGH' showed an increase of mass of 31±2 amu as compared with rhGH. After reduction using DTT, the mass of the rhGH' is identical to the calculated mass for reduced hGH.

The results are shown in the below Table I.

Table I

	ESMS	Calculated
rhGH	22126±2	22125.2
rhGH'	22157±2	-
rhGH' + DTT	22129±2	-

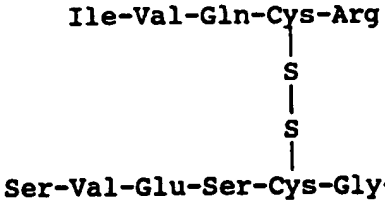
Determination of Mass of the tryptic fragment No. 7 of rhGH and rhGH'

The mass of the tryptic fragment No. 7 of rhGH and rhGH', the 7 and 7' fragments, respectively, were determined by PDMS. The 7 fragment arises from tryptic peptide mapping of rhGH.

hGH in a concentration of 1 mg/ml was dialysed against 50 mM Tris, 2 mM $CaCl_2$, 6 H_2O, pH 7.8 for 24 hours af 4°C. 10 µl of a trypsin solution prepared by dissolving 1 mg trypsin (Bovine, DPCC treated, T-1005 from Sigma) pr. ml. 1 mM HCl, 2 mM $CaCl_2.6H_2O$ was added pr mg. hGH. The digestion was performed in 6 hours at 37°C. The digestion product was analysed (25 µl) using RP-HPLC: Column: Nucleosil RP C18, 250x4 mm, 120 Å, 7u (Macherey-Nagel, Art. 720042.

Temperature: 45°C. Detection : 215 nm. Flow: 1 ml/min. Eluent E: 0.05% (vol/vol) TFA in water, eluent F: 0.05% (vol/vol) TFA in 70% acetonitrile in water. Gradient: 0 to 70% eluent F during 60 minutes. Then the gradient was changed to 100% F during 5 minutes followed by 10 minutes isocratic elution at 100% F. The gradient was changed back to 0% F during 1 minute and the column was equilibrated for 15 minutes before next run.

The 7 fragment of rhGH produced by tryptic cleavage has a calculated mass of 1401 and the Formula I



(I)

Mr: 1401

A difference in mass of 32 amu between the 7 and 7' peptides is observed. After reduction using DTT, identical masses are found for both the 7 and the 7' peptides corresponding to the the calculated mass for the reduced peptide.

The results are shown in the below Table II.

Table II

	PDMS	Calculated
7 fragment	1401	1401
7 fragment + DTT	617 + 785	618 + 785
7'fragment	1433	
7'fragtment + DTT	617 + 785	

The 7' fragment was isolated by collecting the fraction corresponding to the new peak by RP-HPLC of the trypsin digest as discribed above.

A partial Edman Degradation combined with PDMS analysis as well as ESMS was carried out directly on the 7' fragment. Through four steps it was possible to trace the manual degradation by analyzing the truncated peptide. In each step, two amino acid residues were cleaved off (one from each N-terminal). The difference in mass of 32 amu between the 7 and 7' peptides was not changed during these four cleavages.

MS/MS analysis by ESMS gave a series of ionized sequences related to the N-terminal part of the peptide. The MS/MS was carried out using the molecular ion of the 7' peptide having the mass 717amu and a double charge. The fragmentation of the "upper chain" gave rise to peaks at m/z 1320, 1221, and 1094, whereas the fragmentation of the "lower chain" gave rise to peaks at m/z 1247, 1118, 1061, and 974. The conclusion is that the first four amino acid residues in each "chain" - as far as the cystein residues - show normal masses.

Thus, the difference in mass of 32 amu between the rhGH and rhGH' seems to be due to the presence of a trisulphide as opposed to the normal disulphide.

Demonstration of the Presence of Extra Sulphur in hGH'

The presence of a trisulphide bridge was demonstrated using lead acetate as described below.

Treatment of rhGH' with cysteine as described below was demonstrated to transform the rhGH' into native rhGH during which the development of hydrogen sulphide was detected.

Filter paper (Whatman glass microfibre filters) was soaked in a 0.1M solution of lead acetate in distilled water, and air dried.

Six test tubes were prepared having the contents as stated in the below Table III.

Tabel III

2 tubes of 10 ml containing: Water
2 tubes of 10 ml containing: Pure hGH'
2 tubes of 10 ml containing: hGH - without peak 7'

The test tubes were divided into two series as stated in the below Table IV.

Tabel IV

Series I		Series II	
Tube	Containing	Tube	Containing
1	Water	4	Water
2	Pure hGH'	5	Pure hGH'
3	hGH (without peak 7')	6	hGH (without peak 7')

To all tubes of series I was added 2.5 ml of distilled water.

To all tubes of series II was added 2.5 ml of 2.5 mM cysteine in distilled water.

The paper was cut into six pieces (rondels of a diameter of 3.5 cm) and placed at the top of the eight test tubes. The rondels were moistened by adding 3-4 drops of distilled water, and the test tubes were left in a water bath at 40°C for 24 hours.

After 24 hours the paper rondels were examined. No change was seen for the test tubes having had water added.

On the papers on test tubes 4 and 6 having had added cysteine, a very faint brownish colouring was observed.

The paper on test tube No. 5 showed a dense black spot ascribed to the formation of lead sulphide. The black spot appeared after 10 to 15 minutes.

Example

Conversion of Hydrophobic Derivative of Human Growth Hormone into Native Human Growth Hormone

Lyophilized rhGH from a sample comprising rhGH' was diluted with destilled water to a concentration of 1mg hGH/ml. The contents of BhGH' in this preparation was 18%.

A 40 mM solution of sodium sulphite pH 7 was prepared by dissolving 50.4 mg sodium sulphite in 10 ml distilled water.

To 1 ml 40 mM solution of sodium sulphite was added 19 ml distilled water to obtain a 2mM solution of sodium sulphite.

0.5 ml solution of rhGH comprising 1 mg BhGH/ml was mixed with 0.5 ml 2mM solution of sodium sulphite giving a 1mM solution of sulphite and was left for 30 minutes at room temperature.

0.5 ml solution of rhGH comprising 1 mg BhGH/ml was mixed with 0.5 ml 40mM solution of sodium sulphite giving a 20mM solution of sulphite and was left for 30 minutes at room temperature.

After standing, the samples were analyzed by hydrophobic interaction chromatography (HIC) as described in Example A.

The resulting chromatograms of the samples are shown in the Figure as Fig. 1 and Fig 2., respectively. In the sample having a concentration of sodium sulphite of 1mM the contents of BhGH' was reduced to about 16%, whereas the contents og BhGH' was nearly quantitatively removed in the sample containing 20 mM sodium sulphite.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Novo Nordisk A/S
(B) STREET: Novo Alle
(C) CITY: DK-2880 Bagsvaerd
(E) COUNTRY: Denmark
(G) TELEPHONE: +45 44448888
(H) TELEFAX: +45 44490555
(I) TELEX: 37173

(ii) TITLE OF INVENTION: A Method of Converting a Hyrophobic Derivative of a Polypeptide into the Native Form

(iii) NUMBER OF SEQUENCES: 2

(iv) COMPUTER READABLE FORM:
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(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(v) CURRENT APPLICATION DATA:
APPLICATION NUMBER:
(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Ile Val Gln Cys Arg
1 5

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Ser Val Glu Ser Cys Gly Phe
1 5

CLAIMS

1. A method for converting a hydrophobic derivative of a growth hormone into the native form of the growth hormone, wherein the derivative of growth hormone is treated with a sulfite compound.

2. A method as claimed in claim 1, wherein the sulphite compound is an alkalimetal sulphite, ammonium sulfite, or an alkaline-earth metal sulfite.

3. A method as claimed in claim 1, wherein the sulphite is formed in situ by adding sulphur dioxide to a solution of the hydrophobic derivative of growth hormone.

4. A method as claimed in any of claims 1-3, wherein the concentration of the sulphite compound compound is up to a saturated solution.

5. A method as claimed in claim 4, wherein the the sulphite compound is sodium sulphite in a concentration of from 10 to 30 mM.

6. A method as claimed in any of claims 1-5, wherein the pH is adjusted to 3-11.

7. A method as claimed in claim 6, wherein the pH is adjusted to about 7.

8. A method as claimed in any of claims 1-7, wherein the treatment is carried out at a temperature from about 0·C to about 50·C.

9. A method as claimed in claim 8, wherein the temperature is about room temperature.

10. A method as claimed in any of claims 1-9, wherein the growth hormone is human growth hormone.

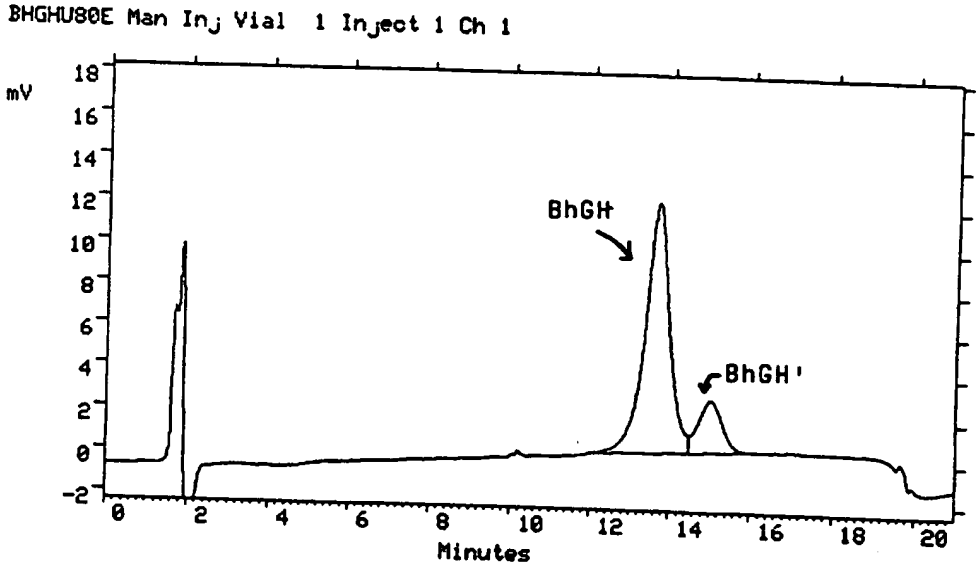
BHGHU80E Man Inj Vial 1 Inject 1 Ch 1
mV
18
16
14
12
10
8
6
4
2
0
-2
0
2
4
6
8
10
12
14
16
18
20
Minutes
BhGH
BhGH'

Fig. 1

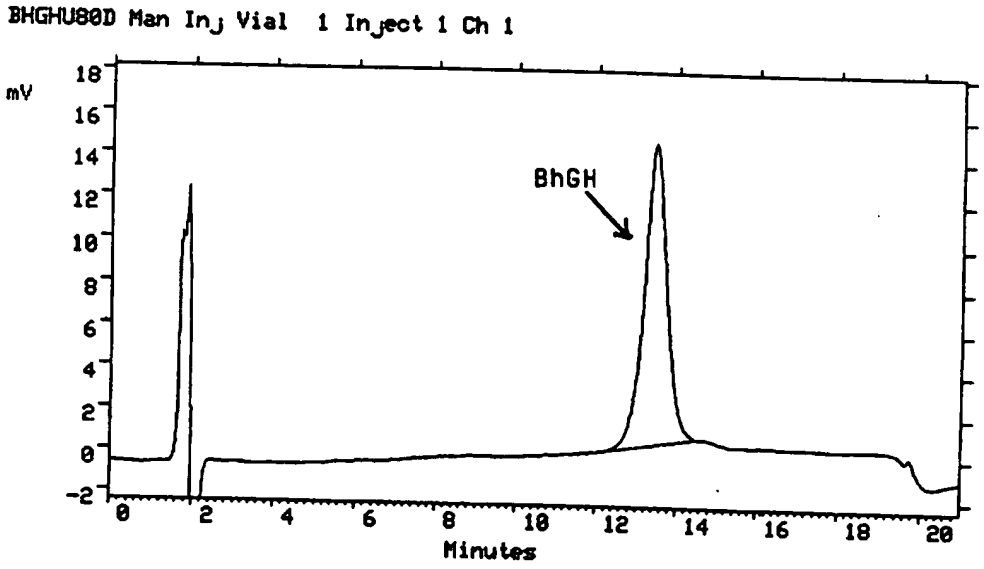
BHGHU80D Man Inj Vial 1 Inject 1 Ch 1
mV
18
16
14
12
10
8
6
4
2
0
-2
BhGH
0
2
4
6
8
10
12
14
16
18
20
Minutes

Fig. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 95/00307

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 14/61, C07K 1/107
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPIDS, IFIPAT, CA, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0361830 A2 (ELI LILLY AND COMPANY), 4 April 1990 (04.04.90), see page 2, lines 19-31; the claims ---	1-10
A	WO 9204382 A (BUNGE (AUSTRALIA) PTY. LTD.), 19 March 1992 (19.03.92) --- --------	1-10

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 October 1995	02 -11- 1995
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carolina Palmcrantz Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

02/10/95

International application No.
PCT/DK 95/00307

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A2- 0361830	04/04/90	JP-A- 2142490	31/05/90
		US-A- 4923967	08/05/90
WO-A- 9204382	19/03/92	AU-A- 8403591	30/03/92
		EP-A,A- 0547102	23/06/93